Reference: *Biol. Bull.*, 138: 211–218. (April, 1970)

OBSERVATIONS ON THE FUNCTION OF THE FRONTOLATERAL HORNS AND HORN GLANDS OF BARNACLE NAUPLII (CIRRIPEDIA)

PETER B. TAYLOR [1]

Department of Oceanography, University of Washington, Seattle, Washington 98105

Frontolateral horns and associated glands are prominent, characteristic features of the nauplii of most Cirripedia. Frontolateral horns are possessed by the orders Thoracica (including the Balanomorpha, Verrucomorpha, and Lepadomorpha), the Acrothoracica, and the Rhizocephala, but not by the Ascothoracida. It is by the frontolateral horns that the nauplii of true cirripeds can be distinguished from those of other Crustacea. They must serve some function, and this paper is concerned with a hypothesis that the frontolateral horns and horn glands constitute venomous organs that serve barnacle nauplii as a defense mechanism against predation.

Balanus perforatus nauplii (Balanomorpha), as described by Groom (1894), have frontolateral horns that are typical of barnacle nauplii in general (Fig. 1). The frontolateral horns and horn glands of the Acrothoracica and the Rhizocephala are essentially similar to those of the Thoracica (Krüger, 1940). The horns are tubular structures projecting from the dorsal shield. Their tips are perforate. Within each horn is a spindle-shaped organ consisting of two closely applied gland cells. The "frontolateral horn glands" extend from a point near the middle of the dorsal shield, above the gut, into the lumen of each horn. Transparent globules of secretion may fill the lumen. Groom (1894, page 193) described the globules, which he said are ". . . provided with a resistant pellicle, and which, though closely pressed against one another into polyhedral bodies, . . . do not fuse; they are not dissolved by water, alcohol, weak acids or alkalies; they show no acid or alkaline reaction and take up no colouring matter." He also speculated (page 193) that the membranes of the glands and of the globules are chitinous, but pointed out that "the chitin . . . must be of a delicate nature, for while resembling the cuticular covering of the whole body in being soluble in warm acid (HNO_3), it dissolves in hot caustic potash, which ordinary cuticle resists."

To my knowledge, Groom's are the most detailed descriptions of the horns, horn glands, and secretion that exist for any cirriped nauplii. Kaufmann (1965), however, presented photomicrographs of the horns and horn glands of *Scalpellum scalpellum*. Maximum development of the horns is attained among the Lepadomorpha, for example in the genus *Lepas* (Groom, 1894, Figure 156).

In the thoracican barnacles, the horns and horn glands are initially formed during later embryonic stages (Groom, 1894; Krüger, 1940). Stage I nauplii have

[1] This research was supported by a National Science Foundation Postdoctoral Fellowship to the author and by grant GB-3551 from the National Science Foundation to the University of Washington. Contribution No. 519 from the Department of Oceanography, University of Washington, Seattle, Washington, and Contribution No. 157 from the Systematics-Ecology Program, Marine Biological Laboratory, Woods Hole, Massachusetts.

the horns directed posteriorly along the lateral margins of the dorsal shield and the tips are closed. Following the first molt and for the five subsequent naupliar stages, the horns are directed anterolaterally and somewhat ventrally. The horn tips are perforate and complex, consisting of terminal stylets and spine processes. The horns appear to become vestigial during metamorphosis of the metanauplius (Stage VI) to the cyprid larval stage. In the cyprids of *Scalpellum scalpellum* they are reduced to closed, short, terminally widened, funnel-shaped stumps into which the glands open (Kaufmann, 1965).

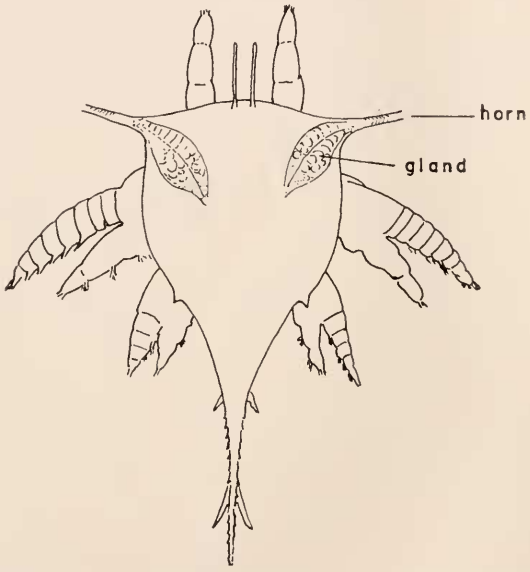

FIGURE 1. The stage II nauplius of *Balanus perforatus* (after Groom, 1894, Fig. 141); setation omitted; Horn = frontolateral horn, Gland = frontolateral horn gland.

Speculations on the function of the frontolateral horns and horn glands were advanced during the nineteenth century. Darwin (1851), regarding the frontal filaments as a pair of appendages, professed to see within the horns the developing second pair (second antennae). Dohrn (1870) considered the horns and their glands as possible precursors of the adhering organs of the cyprid. We know now that the frontal filaments and the horns are not true appendages and that the glands of the true first antennae are the adhering organs.

Groom (1894, page 193) pointed out that in the Thoracica the period of secretion of the horn glands coincides with the free life of the larvae, during part of which they feed. He agreed with Claus and with Hoek (cited by Groom, page 193) that the sharp points and spines at the ends of the horns indicate that they may be piercing organs provided with poison glands. He further observed ". . . that the area covered by the horns is that included by the sweep of the appendages, and that any organism paralyzed by the secretion would tend to be swept towards the region of the mouth." Groom thus proposed a feeding function.

Recently, Drs. W. A. Newman and S. A. Wainwright reported observations

(personal communications) from which they inferred that the frontolateral horns and horn glands might be venomous or noxious organs for defense against predation. Newman observed an increased mortality of shrimp (*Palaemon*) larvae in culture following the introduction of (*Balanus*) nauplii; Wainwright noted the vigorous withdrawal of coral (*Pocillopora*) tentacles from contact with barnacle (*Balanus*) nauplii in laboratory observations. My observations, reported here test the hypothesis of the existence of a venomous defense mechanism. The observations were made at the Marine Biological Laboratory, Woods Hole, Massachusetts, and at the Friday Harbor Laboratories (University of Washington), Friday Harbor, Washington.

Materials and Methods

Barnacle nauplii reared in the laboratory were used for most trials. Rearing methods were based on those of Moyse (1960). Cultures were initiated from embryos removed from the mantle cavities of adult barnacles. Usually 200 to 400 nauplii were reared in each 1-liter glass Berzelius beaker containing paper-filtered or membrane-filtered (Millipore HA 45 μ) seawater. Portions of unialgal cultures of *Skeletonema costatum, Cyclotella nana, Cyclotella cryptica,* and *Thalassiosira fluviatilis* were added for food. Continuous light was provided to maintain algal growth in the rearing vessels. At Woods Hole, the vessels were maintained near 12° C in a constant-temperature room; at Friday Harbor they were kept on a running seawater table at temperatures between 11 and 13° C, near local surface water temperature. The algal cells were resuspended daily by stirring and were replenished if depleted. The *Cyclotella* spp. were the most satisfactory of the algae used because they remained in suspension and grew rapidly at rearing-vessel temperatures. Nauplii were transferred to clean vessels and seawater containing fresh algal culture at least weekly. In this way embryos of *Balanus balanoides, B. balanus, B. improvisus,* and *B. glandula* were reared to cyprids. The success of the culturing methods was further evidenced by the fact that cyprids settled as spat in rearing vessels when subsequently left undisturbed. Attempts to rear *Pollicipes polymerus* by these methods failed beyond Stage II nauplii.

"Wild" barnacle nauplii were isolated from plankton net collections (153-μ-mesh, 30-cm-diameter net) from near the water surface. They were not identified beyond genus.

Brine shrimp (*Artemia salina*) nauplii were hatched and reared in fingerbowls containing filtered seawater plus portions of unialgal cultures, kept under lights at ordinary room temperatures.

Potential predatory species were isolated from plankton-net collections, or were dip-netted by day (or at night under a submerged light); benthic forms were collected intertidally. These animals were generally held in 16-oz (473-ml) low-form plastic containers or glass jars on a seawater table to maintain lower temperatures. *Artemia* nauplii were supplied as food. Planktonic crustaceans seemed to survive best in vessels made dark by enclosure in black plastic.

Observational Methods

The direct approach was to observe the reactions of potential predators to barnacle nauplii. Barnacle nauplii were pipetted into a vessel usually containing

a potential predator. Behavior was observed directly or with the aid of a dissection microscope. For comparison, the reactions of predators to *Artemia* nauplii were observed. These reactions also tested the predators' predisposition to feed before the trials. Stage I barnacle nauplii, presumably "unarmed" because the frontolateral horns were not yet erect or perforate, also served as controls. The warming of animals above maintenance temperatures was minimized by working in a cold room or by observing for only short periods and using large water volumes.

Survival trials were also conducted to avoid the conditions of continuous bright light and handling, which might interfere with the normal behavior of the barnacle nauplii or the potential predators. Usually 10 barnacle nauplii were introduced into a 16-oz (473-ml) low-form wide-mouth glass jar containing a single predator. Predator mortality, the consumption of barnacle nauplii, or other effects were then determined through periodic censuses. *Artemia* nauplii and Stage I barnacle nauplii were used as controls in separate vessels, and were cointroduced with later-stage barnacle nauplii. Still other controls confirmed survival of the nauplii in the vessels without predators. Between censuses, all vessels were kept on a running seawater table for temperature control, and all except those containing medusae were darkened by black plastic enclosures. Censuses of the number and condition of the animals were made under oblique or side lighting against a black background. Predation was best demonstrated by the presence of prey remains in the guts of the predators, or in their fecal material examined under a dissection microscope, because counts of surviving nauplii were difficult to make and did not prove the fate of missing individuals.

Results

Direct observations on the behavior of certain potential predators toward barnacle nauplii are summarized in Table I. Other animals (chaetognaths, euphausids, predatory copepods, decapod zoeae, medusae, and fish postlarvae) were tried but failed to feed under the viewing conditions. Several representatives of the Cnidaria and bony fishes captured and consumed barnacle nauplii, and did so without ill effects. On the other hand, for two hydromedusae, *Nemopsis bachei* and *Aequorea aequorea,* the results shown in Table I are perhaps consistent with the defense hypothesis. Both species were indifferent to contact with the later stages of barnacle nauplii.

Results of the survival experiments are summarized in Table II. *Epilabidocera amphitrites* and *Neomysis rayii* consumed later-stage barnacle nauplii as readily as *Artemia* nauplii. Evidences of feeding by the copepod *E. amphitrites* were the folded but intact exoskeletons of barnacle nauplii appearing in fecal material deposited in the vessels. Nine such remains were found during the trials. *Artemia* nauplii exoskeletons were similarly found. I also noted (21 instances) the empty exoskeletons of barnacle nauplii in the vessels. Under the microscope these appeared to have been cut across the dorsal shield and some were partially crumpled. They were not exuviae from molting since they were never noted in controls or in trials with other predators. I conclude that the copepods probably were cutting the nauplii, extruding or sucking out the contents, and rejecting the

TABLE I

Observed reactions of potential predators to barnacle nauplii

Potential predators		Barnacle nauplii reactions*		
Species	No. of individuals	Source	Stage I	Other stages
		B. balanoides, Woods Hole		
Anthozoan				
Sagartia sp.	1	cultured		+
Hydroid colony				
Coryne sp.	1	wild		+
Hydromedusae				
Nemopsis bacheii	1	cultured		−
		B. glandula, Friday Harbor		
Sarsia tubulosa	1	cultured	+	
	6	cultured		+
	1	wild		+
Aequorea aequorea	1	cultured	+	
	1	cultured		−
Fish postlarvae				
Sebastodes sp.	1	cultured		+
	1	wild		+
Cottidae	1	cultured		+
	1	wild		+

* + = nauplii captured and ingested; − = no reaction, indifference to contact.

empty exoskeletons. Evidences of this behavior with *Artemia* nauplii were not observed. In neither type of prey was the exoskeleton fragmented, whether it was ingested or rejected. In contrast, fecal material from the mysid *Neomysis rayii* produced only fragments of exoskeletons from barnacle and *Artemia* nauplii. These observations indicate the need of caution in attempting to determine feeding habits of animals from their gut contents without knowing their behavior.

TABLE II

*Results of survival experiments**

Potential Predators	*Balanus*		*Pollicipes*	
	Stage I (cultured) +/−	Other stages (cultured) +/−	Other stages (wild) +/−	Other stages (cultured) +/−
Hydromedusae				
Aequorea aequorea	6/2	1/16		1/7
Phialidium gregarium	3/0	2/5		
Sarsia tubulosa	1/0	6/0	2/0	
Copepod				
Epilabidocera amphitrites		19/0	2/0	
Mysid				
Neomysis rayii		10/0	4/0	2/0

* Numerical data = $\frac{\text{number of trials, nauplii consumed (+)}}{\text{number of trials, nauplii not consumed (−)}}$.

Among the hydromedusae the results of the survival experiments for *Aequorea aequorea* were consistent with the direct observations. *Aequorea* consumed Stage I and *Artemia* nauplii, but generally not later-stage barnacle nauplii. These data result from direct examination of the guts of living medusae, which are sufficiently transparent for the stages to be distinguished under low magnification.

Phialidium gregarium consumed barnacle nauplii of all stages but captured them less rapidly than Artemia nauplii, whether introduced together or separately. *Sarsia tubulosa* consumed all prey equally readily. On several occasions one or both frontolateral horns of an ingested barnacle nauplius were seen penetrating the wall of the manubrium without apparent harm.

For reasons mentioned later in the discussion of results, a special attempt was made at Friday Harbor in 1967 to view the reactions between *Balanus* nauplii and specimens of a ctenophore, *Pleurobrachia bachei*. The results were mixed. Poor condition of the *Pleurobrachia* specimens may have been a factor. They were collected during their reappearance following a dramatic decline in availability associated with exceptional warming and lowered salinity in the surface waters of Puget Sound and the Friday Harbor area in June 1967. *Balanus* nauplii likewise were not abundant in the zooplankton. Of the many *Pleurobrachia* specimens used, only a few indicated a readiness to feed by extending their tentacles in gently swirling water. Only three ingested food offered during the observations. One of these captured and ingested *Artemia* nauplii as fast as they were supplied, taking eight within 40 minutes. Of ten *Balanus glandula* nauplii (cultured Stages II and III) supplied, two adhered to tentacles, but they later swam free. Another *Pleurobrachia* specimen took three *Artemia* nauplii; two *Balanus* sp. "wild" nauplii adhered but were not ingested. A third specimen captured and ingested one *Artemia* nauplius and two *Balanus* sp. nauplii ("wild" Stages IV and VI). These and many more observations of contact showed that *Artemia* nauplii adhered more frequently to *Pleurobrachia* tentacles than did *Balanus* nauplii. Differences in mechanical stimuli may be a factor, although the nauplii were comparable in size and swimming rate. Copepods, larger and faster, were more consistently trapped in a tangle of tentacles.

Discussion

The results show that there are animals that can consume barnacle nauplii regardless of the frontolateral horns and horn glands. Indeed, *Balanus* nauplii have been listed among the stomach contents of postlarval fishes (Lebour, 1920; Sanders, 1952; Wailes, 1936) and the medusae *Phialidium hemisphericum* and *Aurelia aurita* (Lebour, 1922; 1923). Hardy and Bainbridge (1954) observed *Aurelia aurita* feeding on *Balanus* nauplii in the laboratory. These reports, however, do not disprove the defense hypothesis, because stage determinations were not mentioned; the nauplii might have been in "unarmed" Stage I. The present observations of the consumption of later-stage barnacle nauplii by a variety of predators and the passiveness of the nauplii to these predators would seem to rule out a generalized use of the horns as a venomous defense mechanism. Such use, however, under more specific circumstances or against a restricted class of predators, is not disproved in view of the results with hydromedusae.

The insolubility of the secretion in aqueous solvents, observed from living and freshly crushed specimens in this study and by Groom (1894), argues further against the secretion of a venom in the sense of a toxin to be introduced into the circulation of a victim.

That the frontolateral horns and horn glands are major features of barnacle nauplii emphasizes their importance. The glands secrete externally and are expected to be involved in behavior or excretion. The feeding function postulated by Groom (1894) is negated by the nonpredatory habits of the nauplii and by the fact that Rhizocephelan nauplii have well-developed horns and horn glands but do not feed. The nonaqueous nature of the secretion does not support involvement in social communication, as an attractant or a repellant, or in excretion. Other possible purposes for the secretion are in metamorphosis or cuticle formation, or in buoyancy adjustment.

The most attractive hypothesis suggested by the present observations is that the nauplii secrete through the horns a surface-active substance that prevents capture by animals that capture prey by use of adhesive organs. Cnidarians, including the hydromedusae, bear a variety of nematocysts, including some that are sticky rather than venomous (Russell, 1953). Dependence on this kind of nematocyst by *Aequorea* or *Phialidium* could explain their pattern of feeding on *Balanus* nauplii. Ctenophores depend on adhesive organs (colloblasts) for capturing prey. The observations on *Pleurobrachia bachei,* although inconclusive, did not support this hypothesis.

A surface-active agent might also prevent entrapment of the nauplii at the sea surface. *Balanus* nauplii are characteristically positively phototactic. In the present work they congregated near the air-water interface of culture vessels lighted from above but did not become trapped at the interface in the way copepods and amphipods often do. The contrast, of course, can be explained by the ability of the latter to penetrate the water surface by vigorous movements.

How barnacle nauplii use the frontolateral horns remains a mystery. A study of the chemical nature of the secretion, probably using histochemical techniques, seems to offer the best possibility for further clues of their function.

I am grateful to Dr. William A. Newman for bringing the problem to my attention and for reading the manuscript. Discussions with Drs. Dora P. Henry and Stephen A. Wainwright were helpful. I wish to thank Dr. Melbourne R. Carriker, Director, Systematics-Ecology Program, Marine Biological Laboratory, Woods Hole, Massachusetts, and Dr. Robert L. Fernald, Director, Friday Harbor Laboratories, University of Washington, for providing accommodations for this research. Miss Dora R. May and Mr. Peter J. Blanton provided technical assistance.

Summary

1. Barnacle nauplii characteristically have frontolateral horns and horn glands, but the function of these is unknown. To examine the hypothesis that they are venomous organs that serve in defense against predation, observations were made on living specimens in the presence of potential predators.

2. Laboratory observations of two general types were made on *Balanus* spp. nauplii. One was to observe directly the behavioral interactions between the nauplii and potential predators. The other involved survival trials, in which barnacle nauplii were introduced into vessels with potential predators; the losses due to predation were recorded at intervals.

3. The results of these observations strongly suggest that barnacle nauplii lack a generalized defense against predation, and there was no indication that the frontolateral horns and horn glands are used as venomous organs. The observed indifference of certain hydromedusae to most contacts with barnacle nauplii suggests, however, that there might be a specialized defense mechanism.

4. Other possible functions of the frontolateral horns are discussed in view of the results.

LITERATURE CITED

Darwin, C., 1851. *A Monograph on the sub-class Cirripedia: The Lepadidae or pedunculated Cirripedes.* Ray Society, London, 400 pp.

Dohrn, A., 1870. Untersuchungen über Bau und Entwicklung der Arthropoden. 9. Eine neue Nauplius-Form (*Archizoëa gigas*). *Z. Wiss. Zool.,* **20**: 597–606.

Groom, T. T., 1894. On the early development of Cirripedia. *Phil. Trans. Roy. Soc. London Series B.,* **185**: 119–232.

Hardy, A. C., and R. Bainbridge, 1954. Experimental observations on the vertical migrations of plankton animals. *J. Mar. Biol. Ass. U.K.,* **33**: 409–448.

Kaufmann, R., 1965. Zur Embryonal- und Larvalentwicklung von *Scalpellum scalpellum* L. (Crust. Cirr.). *Z. Morphol. Tiere,* **55**: 161–232.

Krüger, P., 1940. Cirripedia, 3. Buch, Teil III, pp. 1–560. *In:* H. G. Bronns Klassen und Ordnungen des Tierreichs. Akademische Verlagsgesellschaft, Leipzig, 560 pp.

Lebour, M., 1920. The food of young fish. No. III. *J. Mar. Biol. Ass. U.K.* **12**: 261–324.

Lebour, M., 1922 The food of plankton organisms *J. Mar. Biol. Ass. U.K.,* **12**: 644–677.

Lebour, M., 1923. The food of plankton organisms. II. *J. Mar. Biol. Ass. U.K.,* **13**: 70–92.

Moyse, J., 1960. Mass rearing of barnacle cyprids in the laboratory *Nature,* **185**: 120.

Russell, F. S., 1953. *The Medusae of the British Isles.* Cambridge University Press, 530 pp.

Sanders, H. L., 1952. The herring (*Clupea harengus*) of Block Island Sound. *Bull. Bingham Oceanogr. Collect. Yale Univ.,* **13**: 220–237.

Wailes, G. H., 1936. Food of *Clupea pallasii* in Southern British Columbia waters. *Journal of the Biological Board of Canada,* **1**: 477–486.